

Sequence Listing

<110> Asahi Kasei Kabushiki Kaisha <120> High-concentration preparation of soluble thrombomodulin <130> ASAHI-33 <150>. JP2002-009951 <151> 2002-01-18 <160> 9 <210> 1 <211> 516 <212> PRT <213> Artificial sequence <220> <223> Partial amino acid sequence of human-originated soluble thrombomodulin <400> 1 Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly 10 Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu 20 25 His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala 35 40 45 Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser

					,		_	- 3					-	
50					55					60				
Ser Val	Ala	Ala	Asp	Val	Ile	Ser	Leu	Leu	Leu	Asn	Gly	Asp	Gly	Gly
65				70					75					80
Val Gly	Arg	Arg	Arg	Leu	Trp	Ile	Gly	Leu	Gln	Leu	Pro	Pro	Gly	Cys
			85					90					95	
Gly Asp	Pro	Lys	Arg	Leu	Gly	Pro	Leu	Arg	Gly	Phe	Gln	Trp	Val	Thr
		100					105					110		
Gly Asp	Asn	Asn	Thr	Ser	Tyr	Ser	Arg	Trp	Ala	Arg	Leu	Asp	Leu	Asn
	115	1				120					125			
Gly Ala	Pro	Leu	Cys	Gly	Pro	Leu	Cys	Val	Ala	Val	Ser	Ala	Ala	Glu
130	•			-	135					140				
Ala Thr	Val	Pro	Ser	Glu	Pro	Ile	Trp	Glu	Glu	Gln	Gln	Cys	Glu	Val
145				150					155					160
Lys Ala	Asp	Gly	Phe	Leu	Cys	Glu	Phe	His	Phe	Pro	Ala	Thr	Cys	Arg
			165					170					175	ı
Pro Leu	Ala	Val	Glu	Pro	Gly	Ala	Ala	Ala	Ala	Ala	Val	Ser	Ile	Thr
		180					185					190		
Tyr Gly	Thr	Pro	Phe	Ala	Ala	Arg	Gly	Ala	Asp	Phe	Gln	Ala	Leu	Pro
	195					200					205			
Val Gly	Ser	Ser	Ala	Ala	Val	Ala	Pro	Leu	Gly	Leu	Gln	Leu	Met	Cys
210					215					220	•			
Thr Ala	Pro	Pro	Gly	Ala	Val	Gln	Gly	His	Trp	Ala	Arg	Glu	Ala	Pro
225				230.				-	235					240
Gly Ala	Trp	Asp	Cys	Ser	Val	Glu	Asn	Gly	Gly	Cys	Glu	His	Ala	Cys
			245					250					255	
Asn Ala	Ile	Pro	Glv	Ala	Pro	Ara	Cvs	Gln	Cvs	Pro	Ala	Glv	Ala	Ala

Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys
275
280
285

265

260

270

Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln His Arg Cys Glu Asp Val Asp Cys Ile Leu Glu Pro Ser Pro Cys Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro 355 . Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile Gly Thr Asp Cys 4.75 Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro Pro Ser Pro Thr Pro Gly Ser Thr Leu Thr Pro Pro Ala Val Gly Leu

Val His Ser Gly

515

<210> 2

<211> 1548

<212> DNA

<213> Artificial sequence

<220>

<223> Partial base sequence of human-originated soluble thrombomodulin gene

<400> 2

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gtcaacaca agggtggctt cgagtgccac tgctacccta actacgacct ggtggacggc 1080 gagtgtgtgg agcccgtgga cccgtgcttc agagccaact gcgagtacca gtgccagccc 1140 ctgaaccaaa ctagctacct ctgcgtctgc gccgagggct tcgcgcccat tccccacgag 1200 ccgcacaggt gccagatgtt ttgcaaccag actgcctgtc cagccgactg cgaccccaac 1260 acccaggcta gctgtgagtg ccctgaaggc tacatcctgg acgacggttt catctgcacg 1320 gacatcgacg agtgcgaaaa cggcggcttc tgctccgggg tgtgccacaa cctcccggt 1380 accttcgagt gcatctgcgg gcccgactcg gcccttgtcc gccacattgg caccgac. t 1440 gactccggca aggtggacgg tggcgacagc ggctctggcg agcccccgc cagcccgacg 1500 cccggctcca ccttgactcc tccggccgtg gggctcgtgc attcggc

<210> 3

<211> 132

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble thrombomodulin

<400> 8

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly

1 5 10 15

Phe Pro Asp Pro Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro

20 25 30

Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro

35 40 45

Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala

50 55 60

Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro





65 70 75 80

Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu

90 95

Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly

100 105 110

Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile

115 120 125

Gly Thr Asp Cys

130

<210> 4

<211> 396

<212> DNA

<213> Artificial sequence

<220>

<223> Partial base sequence of human-originated soluble thrombomodulin gene

<400> 4

atgettgggg teetggteet tggegegetg geeetggeeg geetggggtt eeeegaeeeg 60
tgetteagag ceaactgega gtaceagtge cageeeetga accaaactag etacetetge 120
gtetgegeeg agggettege geeeatteee caegageege acaggtgeea gatgtttge 180
aaccagaetg cetgteeage egaetgegae eeeaaceee aggetagetg tgagtgeeet 240
gaaggetaea teetggaega eggttteate tgeaeggaea tegaegagtg egaaaaegge 300
ggettetget eeggggtgtg eeacaaeete eeeggtaeet tegagtgeat etgegggeee 360
gaeteggeee ttgteegeea eattggeaee gaetgt 396

<210> 5



<211> 516

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble thrombomodulin

<400> 5

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly

1 5 10 15

Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu

20 25 30

His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala

35 40 45

Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser

50 55 60

Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly

 65
 70
 75
 80

Val Gly Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys

85 90 95

Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr

100 105 110

Gly Asp Asn Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn

115 120 125

Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu

130 135 140

Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val

145 150 155 160



ьys	Ala	Asp	Gly	Phe	Leu	Cys	Glu	Phe	His	Phe	Pro	Ala	Thr	Cys	Arg
				165					170					175	
Pro	Leu	Ala	Val	Glu	Pro	Gly	Ala	Ala	Ala	Ala	Ala	Val	Ser	Ile	Thr
			180					185					190		
Tyr	Gly	Thr	Pro	Phe	Ala	Ala	Arg	Gly	Ala	Asp	Phe	Gln	Ala	Leu	Pro
		195					200					205			
Val	Gly	Ser	Ser	Ala	Ala	Val	Ala	Pro	Leu	Gly	Leu	Gln	Leu	Met	Cys
	210					215					220				
Thr	Ala	Pro	Pro	Gly	Ala	Val	Gln	Gly	His	Trp	Ala	Arg	Glu	Ala	Pro
225					230					235					240
Gly	Ala	Trp	Asp	Cys	Ser	Val	Glu	Asn	Gly	Gly	Cys	Glu	His	Ala	Суѕ
				245					250					255	
Asn	Ala	Ile	Pro	Gly	Ala	Pro	Arg	Cys	Gln	Cys	Pro	Ala	Gly	Ala	Ala
			260					265					270		
Leu	Gln	Ala	Asp	Gly	Arg	Ser	Cys	Thr	Ala	Ser	Ala	Thr	Gln	Ser	Cys
								-							-
		275					280					285			-
Asn	Asp				His	Phe		Val	Pro	Asn	Pro		Gln		
Asn	Asp 290					Phe 295	Cys	Val	Pro	Asn	Pro 300		Gln		
	290	Leu	Cys	Glu		295	Cys				300	Asp		Pro	Gly
	290	Leu	Cys	Glu	His	295	Cys				300 Leu	Asp		Pro Asp	Gly
Ser 305	290 Tyr	Leu Ser	Cys Cys	Glu Met	His Cys	295 Glu	Cys	Gly	Tyr	Arg 315	300 Leu	Asp Ala	Ala	Pro Asp	Gly Gln 320
Ser 305	290 Tyr	Leu Ser	Cys Cys	Glu Met	His Cys 310	295 Glu	Cys	Gly	Tyr	Arg 315	300 Leu	Asp Ala	Ala	Pro Asp	Gly Gln 320 Cys
Ser 305 His	290 Tyr Arg	Leu Ser Cys	Cys Cys Glu	Glu Met Asp 325	His Cys 310	295 Glu Asp	Cys Thr Asp	Gly Cys	Tyr Ile 330	Arg 315 Leu	300 Leu Glu	Asp Ala Pro	Ala	Pro Asp Pro 335	Gly Gln 320 Cys
Ser 305 His	290 Tyr Arg	Leu Ser Cys	Cys Cys Glu	Glu Met Asp 325	His Cys 310 Val	295 Glu Asp	Cys Thr Asp	Gly Cys	Tyr Ile 330	Arg 315 Leu	300 Leu Glu	Asp Ala Pro	Ala	Pro Asp Pro 335	Gly Gln 320 Cys
Ser 305 His	290 Tyr Arg	Leu Ser Cys	Cys Cys Glu Cys 340	Glu Met Asp 325 Val	His Cys 310 Val	295 Glu Asp	Cys Thr Asp Gln	Gly Cys Gly 345	Tyr Ile 330 Gly	Arg 315 Leu Phe	300 Leu Glu Glu	Asp Ala Pro Cys	Ala Ser His	Pro Asp Pro 335 Cys	Gly Gln 320 Cys
Ser 305 His	290 Tyr Arg Gln Asn	Leu Ser Cys	Cys Cys Glu Cys 340	Glu Met Asp 325 Val	His Cys 310 Val	295 Glu Asp	Cys Thr Asp Gln	Gly Cys Gly 345	Tyr Ile 330 Gly	Arg 315 Leu Phe	300 Leu Glu Glu	Asp Ala Pro Cys	Ala Ser His	Pro Asp Pro 335 Cys	Gly Gln 320 Cys
Ser 305 His Pro	290 Tyr Arg Gln Asn	Leu Ser Cys Arg Tyr 355	Cys Glu Cys 340 Asp	Glu Met Asp 325 Val	His Cys 310 Val	295 Glu Asp Thr	Cys Thr Asp Gln Gly 360	Gly Cys Gly 345 Glu	Tyr Ile 330 Gly Cys	Arg 315 Leu Phe Val	300 Leu Glu Glu	Asp Ala Pro Cys Pro 365	Ala Ser His 350 Val	Pro Asp Pro 335 Cys	Gly Gln 320 Cys Tyr Pro
Ser 305 His Pro	290 Tyr Arg Gln Asn	Leu Ser Cys Arg Tyr 355	Cys Glu Cys 340 Asp	Glu Met Asp 325 Val	His Cys 310 Val Asn	295 Glu Asp Thr	Cys Thr Asp Gln Gly 360 Tyr	Gly Cys Gly 345 Glu	Tyr Ile 330 Gly Cys	Arg 315 Leu Phe Val	300 Leu Glu Glu	Asp Ala Pro Cys Pro 365	Ala Ser His 350 Val	Pro Asp Pro 335 Cys	Gly Gln 320 Cys Tyr Pro

385 390 395 400

Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp

405 410 415

Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile

420 425 430

Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly

435 440 445

Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys

450 455 460

Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile Gly Thr Asp Cys

465 470 475 480

Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro

485 490 495

Pro Ser Pro Thr Pro Gly Ser Thr Leu Thr Pro Pro Ala Val Gly Leu

500 505 510

Val His Ser Gly

515

<210> 6

<211> 1548

<212> DNA

<213> Artificial sequence

<220>

<223> Partial base sequence of human-originated soluble

thrombomodulin gene

<400> 6

atgcttgggg tcctggtcct tggcgcgctg gccctggccg gcctggggtt ccccgcaccc



gcagagccgc agccgggtgg cagccagtgc gtcgagcacg actgcttcgc gctctacccg 120 ggccccgcga ccttcctcaa tgccagtcag atctgcgacg gactgcgggg ccacctaatg 180 acagtgcgct cctcggtggc tgccgatgtc atttccttgc tactgaacgg cgacggcggc 240 gttggccgcc ggcgcctctg gatcggcctg cagctgccac ccggctgcgg cgaccccaag 300 cgcctcgggc ccctgcgcgg cttccagtgg gttacgggag acaacaacac cagctatagc 360 aggtgggcac ggctcgacct caatggggct cccctctgcg gcccgttgtg cgtcgctgtc 420 teegetgetg aggecactgt geccagegag cegatetggg aggageagea gtgegaagtg 480 aaggccgatg gcttcctctg cgagttccac ttcccagcca cctgcaggcc actggctgtg 540 gageceggeg cegeggetge egeegteteg ateacetaeg geacecegtt egeggeeege 600 ggagcggact tecaggeget geeggtggge ageteegeeg eggtggetee eeteggetta 660 cagctaatgt gcaccgcgcc gcccggagcg gtccaggggc actgggccag ggaggcgccg 720 ggegettggg actgeagegt ggagaaegge ggetgegage aegegtgeaa tgegateeet 780 ggggctcccc gctgccagtg cccagccggc gccgccctgc aggcagacgg gcgctcctgc 840 accgcatccg cgacgcagtc ctgcaacgac ctctgcgagc acttctgcgt tcccaacccc 900 gaccagccgg gctcctactc gtgcatgtgc gagaccggct accggctggc ggccgaccaa 960 caccggtgcg aggacgtgga tgactgcata ctggagccca gtccgtgtcc gcagcgctgt 1020 gtcaacacac agggtggctt cgagtgccac tgctacccta actacgacct ggtggacggc 1080 gagtgtgtgg agcccgtgga cccgtgcttc agagccaact gcgagtacca gtgccagccc 1140 ctgaaccaaa ctagctacct ctgcgtctgc gccgagggct tcgcgcccat tccccacgag 1200 ccgcacaggt gccagatgtt ttgcaaccag actgcctgtc cagccgactg cgaccccaac 1260 acccaggeta getgtgagtg ceetgaagge tacateetgg aegaeggttt catetgeaeg 1320 gacatcgacg agtgcgaaaa cggcggcttc tgctccgggg tgtgccacaa cctccccggt 1380 acettegagt geatetgegg geeegaeteg geeettgeee geeacattgg cacegaetgt 1440 gactceggca aggtggacgg tggcgacagc ggctctggcg agcccccgcc cagcccgacg 1500 cccggctcca ccttgactcc tccggccgtg gggctcgtgc attcgggc 1548

<210> 7

<211> 132

<212> PRT

<213> Artificial sequence

<220>

<223> Partial amino acid sequence of human-originated soluble thrombomodulin

<400> 7

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly

1 5 10 15

Phe Pro Asp Pro Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro

20 25 30

Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro

35 40 45

Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala

50 55 60

Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro

65 70 75 80

Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu

85 90 95

Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly

100 105 110

Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile

115 120 125

Gly Thr Asp Cys

130

<210> 8

<211> 396

<212> DNA

<213> Artificial sequence





<220>

<223> Partial base sequence of human-originated soluble thrombomodulin gene

<400> 8

atgcttgggg	tcctggtcct	tggcgcgctg	gccctggccg	gcctggggtt	ccccgacccg	60
tgcttcagag	ccaactgcga	gtaccagtgc	cagcccctga	accaaactag	ctacctctgc	120
gtctgcgccg	agggcttcgc	gcccattccc	cacgageege	acaggtgcca	gatgttttgc	180
aaccagactg	cctgtccagc	cgactgcgac	cccaacaccc	aggctagctg	tgagtgccct	240
gaaggctaca	tcctggacga	cggtttcatc	tgcacggaca	tcgacgagtg	cgaaaacggc	300
ggcttctgct	ccggggtgtg	ccacaacctc	cccggtacct	tcgagtgcat	ctgcgggccc	360
gactcggccc	ttgcccgcca	cattggcacc	gactgt			396

<210> 9

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic DNA for mutation

<400> 9

aatgtggcgg gcaagggccg a

21